PREVALENCE OF β2 GLYCOPROTEIN 1 DEPENDENT ANTICARDIOLIPIN ANTIBODIES IN ACUTE ISCHEMIC STROKE

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the regulations for the award of the degree of

M.D. BRANCH I

GENERAL MEDICINE

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI, INDIA

MARCH 2008

CERTIFICATE

This is to certify that the dissertation titled **"PREVALENCE OF β2 GLYCOPROTEIN 1 DEPENDENT ANTICARDIOLIPIN ANTIBODIES IN ACUTE ISCHEMIC STROKE"** is the bonafide original work of **DR.N.JAYANTHI** in partial fulfillment of the requirements for M.D. Branch – I (General Medicine) Examination of the Tamilnadu DR. M.G.R Medical University to be held in MARCH 2008. The Period of study was from July 2006 and March 2007.

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DECLARATION

I, **DR.N.JAYANTHI,** solemnly declare that dissertation titled **"PREVALENCE OF β2 GLYCOPROTEIN 1 DEPENDENT ANTICARDIOLIPIN ANTIBODIES IN ACUTE ISCHEMIC STROKE"** is a bonafide work done by me at Government Stanley Medical College and Hospital during July 2006 to March 2007 under the guidance and supervision of my unit chief **Prof. Dr. K. RAJENDRAN, M.D.,** Professor of Medicine, Government Stanley Medical College and Hospital, Chennai.

This dissertation is submitted to Tamilnadu DR. M.G.R Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree (Branch – I) in General Medicine – March 2008.**

Place : Chennai.

Date : **(DR. N. JAYANTHI)**

ACKNOWLEDGEMENT

I owe my thanks to the Dean, Govt. Stanley Medical College and Hospital, **Dr. MYTHILI BHASKARAN, M.D.,** for allowing me to avail the facilities needed for my dissertation work.

I am grateful to **Prof. S. NATARAJAN, M.D.,** Professor and Head of the Department of Medicine, Govt. Stanley Medical College and Hospital for permitting me to do the study and for his encouragement.

I express my gratitude to **Prof. K. RAJENDRAN, M.D.,** Professor of Medicine and Chief of Medical Unit VI, Government Stanley Medical College & Hospital for his valuable assistance and guidance.

I owe my sincere thanks to the Professor of Immunology, **Prof. SHANTHA, M.D., Ph.D.,** Govt. Stanley Medical College, for her guidance and help during this study.

I am extremely thankful to my Assistant Professors **Dr. SURENDRAN, M.D.,** and **Dr.SAMUEL DINESH, M.D.,** for their guidance and encouragement.

I am grateful to **Dr. M. PAZHANIVEL, M.D., D.M. (MGE)** for his encouragement, guidance and help during this study.

Last but not the least, my sincere thanks to all the patients who co-operated for this study.

CONTENTS

INTRODUCTION

Acute Ischemic Stroke is one of the leading causes of death and disability in developed nations and is increasing rapidly in the developing world. The prevalence of cerebrovascular disease has progressively increased in India during the last half century. Therefore, an aggressive outlook for the evaluation of cerebrovascular disease is required for some special risk factors apart from the conventional risk factors.

Antiphospholipid antibodies are a heterogenous family of autoantibodies that are directed against the negatively charged phospholipids such as cardiolipin, phosphatidyl serine, phophatidyl inositol.

The antiphospholipid antibodies, which are directed against cardiolipin, have been associated with accelerated atherosclerosis, which is the pathophysiological basis for an ischemic stroke. They have been implicated as one of the most common acquired protein defects causing thrombosis[1].

The binding of antibodies to cardiolipin requires a cofactor. The β2 glycoprotein also known as apolipoprotein H is a highly glycosylated plasma protein that appears to be the major, but not the only cofactor for the recognition of cardiolipin. Other Cofactors are prothrombin, Protein C, Protein S and thrombomodulin. The presence of β2GP1 dependent antibodies was shown to be more specific for thrombosis than non-β2 GP1 dependent anticardioloipin antibodies.

Due to the unexplained aetiology in large number of young stroke patients and absence of conventional predisposing risk factors like HT, DM, Dyslipidemia the importance of antiphospholipid antibodies increased many fold.

In India, a few studies have been conducted regarding the prevalence of anti cardiolipin antibodies.

AIM OF THE STUDY

1. To find out the prevalence of β2 – Glycoprotein 1 Dependent Anticardiolipin Antibodies (β2 GP1) of isotype IgG and IgM in patients with acute ischemic stroke who were 55 years old (or) younger.

2. To find out the correlation between the conventional cerebrovascular risk factors and the anticardiolipin antibodies.

REVIEW OF LITERATURE

Among all the neurological diseases of adult life, the cerebrovascular ones clearly rank first in frequency and importance. After the heart disease and cancer, it is the third most common cause of death in the United States. In the last decade, according to the American Heart Association, the total number of strokes may again be rising[2].

THE CAUSES OF CEREBRAL ISCHEMIA AND INFARCTION

Arterial wall disorders

- Atherothromboembolism
- Intracranial small vessel disease (lipophyalinosis, arteriolosclerosis, microatheroma)
- Trauma
- Dissection
- Fibromuscular dysplasia
- Congenital arterial anomalies
- Moyamoya syndrome
- Embolism from arterial aneurysms
- Inflammatory vascular diseases
- Irradiation
- Infections

Embolism from the heart

Haematological disorders

Miscellaneous conditions

- Pregnancy/puerperium
- Oral contraceptives and other female sex hormones
- Drug abuse
- Cancer
- Perioperative
- Migraine
- Inflammatory bowel disease
- Homocystinaemia
- Fabry's disease
- Mitochondrial cytopathy
- Hypoglycaemia
- Fibrocartilaginous embolism
- Snake bite
- Fat embolism
- Epidermal naevus syndrome
- Nephrotic syndrome
- CADASIL

Most cerebrovascular diseases can be attributed to atherosclerosis. It is more likely to occur in patients with certain risk factors for this disease. Despite the fact that most cerebrovascular events are explained by the conventional risk factors, the search for additional etiologic agents continues. In recent years a number of new candidate risk factors (or) markers have been proposed as significant predictors of atherosclerosis and its complications.

MAJOR INDEPENDENT RISK FACTORS

- Advancing age
- Tobacco smoking
- Diabetes Mellitus
- Elevated total and low-density lipoprotein
- Hypertension

PREDISPOSING RISK FACTORS

- Abdominal obesity
- Ethnic atherosclerosis
- Family history of premature coronary heart disease
- Obesity
- Physical inactivity
- Psychosocial factors

NOVEL RISK FACTORS FOR ATHEROSCLEROTIC VASCULAR DISEASE[3]

1. Inflammatory markers

- C-reactive protein
- Interleukins (eg. IL-6)
- Serum Amyloid A
- Vascular and cell adhesion molecules
- Soluble CD 40 ligand
- Leukocyte count
- Hemostasis / Thrombosis markers
- Fibrinogen
- Von willebrand factor antigen
- Plasminogen activator inhibitor 1 (PAI-1)
- Tissue plasminogen activator
- D-dimer
- Fibrinopeptide A
- Prothrombin fragment
- Platelet related factors
- Platelet aggregation
- Platelet activity
- Platelet size and volume

2. Lipid-related factors

- Small dense low-density lipoprotein
- Lipoprotein (a)
- Remnant lipoproteins
- Apolipoproteins A1 and B
- High-density lipoprotein subtypes
- Oxidized LDL

3. Other factors

- Homocysteine
- Lipoprotein-associated phospholipase A(2)
- Micro albuminuria
- Insulin resistance
- PAI-1 genotype
- Angiotensin-converting enzyme genotype
- ApoE genotype
- Infectious agents – cytomegalaovirus, Chlamydia pneumonia, Helicobacter pylori, Herpes simplex virus

ATHEROTHROMBOEMBOLISM AND CEREBRAL ISCHEMIA [4]

Atheroma seems to be an almost inevitable accompaniment of ageing, at least in developed countries. It is by far the most common arterial disorder and, when complicated by thrombosis or embolism, is the most frequent, but by no means only, cause of cerebral ischaemia and infarction.

The approximate relative frequency of the main causes of ischaemic stroke and TIA

Atherothrombosis affecting large and medium-sized arteries between the heart and the brain	50%
Intracranial small vessel disease (small vessel disease lipophyalinosis / microatheroma, etc.)	25%
Embolism from the heart	20%
Miscellaneous rare disorders	5%

Distribution of atheroma

Atheroma mainly affects large (e.g. aortic arch) and medium sized arteries at places of arterial branching (e.g. carotid bifurcation), tortuosity (e.g. carotid siphon), and confluence (e.g.basilar artery). These are sites of haemodynamic sheer stress and thus endothelial trauma; boundary layer separation, blood stagnation, and the accumulation of platelets; and of turbulence, all of which are likely to promote thrombosis. This might explain the distribution of atheroma in the cerebral circulation if thrombosis is intimately involved with its progression, even if not in its very beginnings. It is remarkable how free of atheroma some sites can be: for example, the internal carotid artery (ICA) between the commonly affected origin and less commonly affected siphon, and the main cerebral arteries distal to the circle of Willis. However, in the same individual, atheroma in one place does tend to be accompanied by atheroma in other parts of the same artery, with atheroma in other arteries to the brain, and in arteries to other organs such as the heart. Presumably this reflects individual susceptibility to atheroma as a result of the presence of causal vascular risk factors (such as hypertension) and genetic

predisposition which determines who will develop atheroma, while the arterial anatomy determines where the lesions occur. None the less, it is curious how severely one arterial site can be affected and yet, in the same individual, the mirror-image site on the other side of the body is still normal, perhaps because of subtle asymmetries in arterial geometry.

Natural history of atheroma

Atheroma starts in childhood, it is thought in response to endothelial injury. Intimal fatty streaks appear first. In a gradual process stretching over many years, circulating monocyte-derived macrophages adhere to and invade the arterial wall, there is an inflammatory response with cytokine production and T-lymphocyte activation, intra and later extracellular cholesterol and other lipids are deposited, particularly in macrophages which are then described as foam cells, smooth muscle cells proliferate, fibrosis occurs, and so fibrolipid plaques are formed. Necrosis and calcification complicate advanced lesions. These atheromatous plaques invade the media, gradually spread around and along the arterial wall and narrow the lumen, although at times the vessel dilates. The plaques are complicated by platelet adhesion, activation, and aggregation, which initiates blood coagulation and subsequent thrombosis.

Thrombus may be incorporated in to the atheromatous plaques which then re-endothelialize; it may grow to obstruct the arterial lumen and then propagate proximally or distally in the stagnant column of blood as far as the next branching point or beyond; it may be lysed by natural fibrinolytic mechanisms in the vessel wall; or it may embolize in whole or in part to occlude a distal artery, usually at a branching point. Such artery-to-artery

emboli vary in size and shape, and consist of some combination of cholesterol debris from the atheromatous plaque, platelet aggregates, and fibrin, which may be newly formed and relatively friable, or old and well organized. Depending on local blood flow and on the size, composition, and consistency of the impacted emboli, they may be lysed, fragment, and vanish into the microcirculation, or remain to occlude the artery and promote local thrombosis. Thrombosis is further encouraged by the release from platelets of thromboxane A_2, which is also a vasoconstrictor. However, it is opposed by prostacyclin and nitric oxide, both vasodilators, released from vascular endothelium, as well as by endothelium derived plasminogen activator. The balance of pro and antithrombotic factors determines whether a thrombus complicating an atheromatous plaque or an occlusive embolus, grows, is lysed, or is incorporated into the vessel wall.

It is likely that atheromatous plaques become ‘active’ or ‘unstable’ from time to time as a result of fissuring and cracking of thin parts of the fibrous cap which covers the rather rigid lesion; of ulceration perhaps; or sometimes of haemorrhage within the plaque, rather than the more commonly found haemorrhage entering via a crack in the endothelial surface. Any of these events exposes the highly thrombogenic necrotic core of the plaque to blood and so causes thrombus to form and then perhaps to embolize. Thus, atherothromboembolism can be regarded as an acute on chronic disease; at any one time a plaque may be static and quiescent with a thick fibrous cap, slowly growing but asymptomatic, or active with ongoing thrombosis and embolization, which may or may not be symptomatic depending on the depth and duration of the consequent ischaemia. This concept may explain the

tendency for TIAs to cluster, for stroke to occur early after a TIA and to affect the same arterial territory, for presumed artery-to-artery embolic strokes to recur early, and for the risk of stroke to decline with time even distal to a severe symptomatic stenosis.

Complicated atheromatous lesions which eventually become fibrotic and heavily calcified make the whole artery rigid, elongated and so tortuous, and sometimes ectatic. Ectasia and aneurysmal bulging, particularly of the basilar artery, may compress adjacent structures, such as the lower cranial nerves and brainstem. Also, emboli may be released from the atheromatous walls and complicating thrombosis. However, arterial rupture is exceptional.

Atherothrombotic plaques are clearly highly dynamic lesions, progressing and regressing in various parts of the arterial tree at different rates and at different times, usually showing layers of thrombus of different ages. Progression and regression of these atherothrombotic lesions can, to some extent, be followed non invasively in humans with ultrasound imaging, but is difficult to distinguish atheroma, which is likely to change only slowly, from thrombus, which may grow or be lysed much faster. Also, intraplaque haemorrhage presumably may cause quite sudden enlargement of a plaque, followed by fairly rapid shrinkage as the haematoma is absorbed. Recently it has become possible to monitor the release of emboli from carotid plaques, and elsewhere, into the cerebral circulation with transcranial Doppler of the middle cerebral artery, again an indication of plaque ‘instability’. Not surprisingly, high intensity embolic signals are more common distal to symptomatic compared with asymptomatic stenosis, but even then the number is rather low, perhaps because of technical difficulties or insufficient recording time.

Symptoms of focal ischaemia occur as a consequence of reduced blood flow which, in the context of aatheroma, is most commonly due to embolism from a plaque complicated by thrombosis in an extracranial artery (such as the carotid bifurcation) to occlude a smaller intracranial artery (such as the mainstem or branch of the middle cerebral artery (MCA)). The notion that the carotid siphon might filter out emboli en route to the brain is interesting but unproven. Occasionally emboli may reach the brain via the collateral circulation; for example from a stenosed internal carotid artery (ICA) across the circle of Willis into the MCA distal to an occluded contralateral ICA; from thrombus in the blind proximal stump of an occluded contralateral ICA, a stenosed proximal external carotid artery (ECA) or from more proximal sites of atheroma, but all via the ECA and through the ophthalmic circulation to the carotid siphon and beyond. Also, emboli may arise from the distal end of a thrombus occluding the ICA. Finally, ischaemia may occur due to haemodynamic compromise beyond an occluded ICA. Furthermore, focal ischaemia may occur between arterial territories (i.e. in boundary zones) usually due to low flow distal to an occluded artery.

Symptomatic in situ atherothrombotic occlusion does not appear to be very common in the anterior cerebral circulation, perhaps because the most commonly affected site for atheroma is in a relatively large artery (i.e. the ICA origin) rather than in smaller arteries, such as the MCA, which are more often occluded by embolism than by in situ atherothrombosis. Another reason might be the relatively effective collateral circulation distal to any occlusion in the extracranial carotid system. On the other hand, symptomatic atherothrombotic occlusion does appear to be more common in the posterior circulation,

particularly in the basilar artery. But even here, embolism from non-occlusive thrombus in to smaller arteries supplying the brainstem and elsewhere is well described.

In an individual patient it is relatively easy to diagnose acute focal ischaemia or infarction but, because angiography is seldom done early, or at all, it is difficult to know what the pattern of any arterial pathology is and exactly how the ischaemia has occurred. Even when angiography, or (increasingly often) ultrasound of the extra and intracranial circulation, reveals an occluded artery, this does not necessarily mean that the occlusion was recent, or that it was embolic rather than due to in situ thrombosis, or whether any embolism had occurred via collaterals or from the distal end of the occluded thrombus, or whether the ischaemia was due to low flow distal to an old occlusion. So, it is now clear that occluded arteries can recanalize spontaneously quite quickly, particularly the mainstem of the MCA. Possibly emboli from the heart are more likely to lyse than those from atheromatous arteries, and thrombotic occlusion of the MCA or ICA is perhaps less likely to open spontaneously. Recanalisation rates presumably also depend on the constituents and age of the occluding material. However, whatever the exact cause of the ischaemia, any demonstrated arterial pathology in the aorta, neck, basilar artery, or circle of Willis is usually atherothrombosis and the assumption is then reasonably made that thromboembolism has occurred at some stage and is likely to occur again. If, as is so often the case, little or no arterial pathology is demonstrated by vascular imaging, or macroscopically at postmortem, then focal ischaemia is most likely due to embolism from the heart or to intracranial small vessel disease.

American Heart Association Classification (1995) of Human Atherosclerosis

Types	Main Histology	Main Pathogenesis	Age Tonset	Clinical
Type I : Initial lesions	Macrophages, occasional foam cell	Accumulation of liporotein	Ist decade	Asympotomatic
Type II : Fatty streaks	Many layers of macrophages and foam cells	Accumulation of liporotein	Ist decade	Asymptomatic
Type III : Intermediate lesions	Many lipid-laden cells and scattered extracellular lipid droplets	Accumulation of liporotein	3rd decade	Asymptomatic
Type IV : Atheromatous lesions	Intra-as well as extra-cellular lipid pool	Accumulation of liporotein	3rd decade	Asymptomatic or manifest symptoms
Type V : Fibrofatty lesions	Fibrotic cap and lipid core (Va), may have calcification (V b)	Smooth muscle cell proliferation and increased collagen	4th decade	Asymptomatic or manifest symptoms
Type VI : Complicated lesions	Ulceration, haemorrhage, haematoma, thrombosis	Haemodynamic stress, thrombosis, haematoma	4th decade	Asymptomatic or manifest symptoms

Atherosclerosis is a complex and indolent histopathological process, which is considered to be the most common underlying process in cerebrovascular disease. In recent years it has become apparent that in addition to the traditional risk factors for atherosclerosis, this condition is associated with infectious, inflammatory and autoimmune factors[5]. Atherosclerosis fulfills all the four criteria delineated by Witebsky and Rose to define a condition as autoimmune in nature and all arms of the immune system (including cellular components, auto antigens and auto antibodies) play a part in atherosclerosis.[6]

INVOLVEMENT OF CELLULAR COMPONENTS IN ATHEROSCLEROSIS

There is good evidence to suggest that cellular components of the immune system are involved in atherosclerosis. Studies using transgenic murine models show recruitment of mononuclear leucocytes through vascular leucocyte adhesion molecules and chemokines, differentiation of monocytes to macrophages, and endocytosis through scavenger receptors of oxidized low-density lipoprotein in atherogenesis. The importance of T cells in atherosclerosis is emphasized in a study in which CD4+ and CD8+ T cell depletion reduced fatty streak formation in C57BL/6 mice, indicating that T cells aggravate fatty streak formation.[7] A recent study emphasizes also the importance of specific lymphocytes. Lymphocytes obtained from low-density lipoprotein receptor deficient mice immunized with β2 glycoprotein were transferred intraperitoneally into syngenic mice, producing larger fatty streaks in the recipients than in mice receiving lymphocytes from the control mice.[8] T cell depletion of lymphocytes failed to induce this effect. Hence, β2 glycoprotein reactive T cells could promote atherogenesis.

It is also of interest to determine whether atherosclerosis is mainly a T helper 1 or T helper 2 mediated conditions. Although it is not yet sufficiently clear, a recent study favors the former.

ASSOCIATION BETWEEN AUTOANTIBODIES AND ATHEROSCLEROSIS

Apart from the involvement of cellular components in atherosclerosis, the evidence also suggests an association between autoantibodies and atherosclerosis (reviewed by Shoenfeld et al[9]). The major antigenic targets for

auto antibodies during atherogenesis are the oxidized lipids such as the oxidized low-density lipoprotein, heat shock proteins (hsp) 60/65, and phospholipids such as cardiolipin[10, 11, 12]

Anti-oxidized low-density lipoprotein antibodies

Oxidation of low-density lipoprotein probably has an important role in the pathogenesis of atherosclerosis. It is not yet firmly established whether the immune response to oxidized low-density lipoprotein is pro-atherogenic or anti atherogenic in vivo, or alternatively, whether an epiphenomenona for the presence of oxidized low-density lipoprotein. Anti-oxidized low-density lipoprotein antibodies are raised in patients with early onset peripheral vascular disease, severe carotid atherosclerosis,[13] and angiographically verified coronary artery disease.[14, 15]

Anti-heat shock protein (HSP) 60/65 antibodies

Heat shock proteins are a family of proteins that shows a highly homologous sequence between different species from bacteria to man. Sonographic assessment of carotid atherosclerotic lesions showed that subjects with such lesions had significantly raised levels of anti-heat shock protein 65 antibodies compared with controls.[16] Early atherosclerosis was significantly enhanced in mice fed high cholesterol that was immunized with heat shock protein 65. Recently it has been suggested that IL4 has a crucial role in the progression of early atherosclerosis mediated by inflammation, as IL4 knockout mice immunized with heat shock protein 65 had significantly less fatty streak formation that lesions in C57BL/6 mice.[17]

ANTINUCLEAR ANTIBODIES IN ATHEROSCLEROSIS

Grainger and Bethel provided evidence for the presence of antinuclear antibodies in patients with radiological evidence of advanced atherosclerosis. Their discussion includes the possibility that these antibodies are merely an epiphenomenona or, alternatively, that they have a pathogenic role in ahterosclerosis.[18] Even though this study naturally does not provide answers to that crucial question, their finding itself is important and raises several thoughts and assumptions.

The accelerated atherosclerotic state found in patients with systemic lupus erythematosus and antiphospholipid syndrome might result from the higher frequency of traditional risk factors in these patients as well as the presence of anticardiolipin and anti-β2glycoprotein1 antibodies.[19] However, antinuclear aantibodies might also contribute to the accelerated atherosclerosis found in these patients. Further, the association of antinuclear antibodies with atherosclerosis raises the possibility that these antibodies play a part in atherogenesis of arteriosclerosis in other autoimmune and inflammatory states, such as vasculitis. As for other antibodies, the frequency of antinuclear antibodies is significantly higher in the elderly people (10 – 37%) than in the young (0 – 6%).[20]

ANTIPHOSPHOLIPID ANTIBODIES IN ATHEROSCLEROSIS

Anticardiolipin antibodies have been associated with accelerated atherosclerosis. They are a heterogenous family of auto antibodies that are directed against the negatively charged phospholipids (such as cardiolipin, phosphatidyl serine, phosphatidyl inositol), Phospholipid-protein complexes, or plasma proteins (such as β2-glycoprotein).[21]

The concept of a protein target for antiphospholipid antibodies evolved from a series of independent reports in 1990. It became clear that the binding of the antibodies to cardiolipin required a cofactor, which was subsequently identified as β2 glycoprotein 1 also known as apolipoprotein H[22,23,24] β2 glycoprotein 1 is a highly glycosylated single chain plasma protein composed of 326 amino acids with a molecular weight of 50 kDa that appears to be the major, but not the only cofactor for the recognition of anionic phospholipid by antiphospholipid antibodies. The protein is a member of the complement control protein or short consensus repeat super family. There is evidence that β2GP1 itself may be one of the major epitopes for antiphospholipid antibodies or may, in complex with phospholipids, form an antigenic site. The physiologic function of β2GP1 has not yet been established, but it has been proposed that the protein may play a scavenging role for exposed anionic phospholipid after apoptosis.[25, 26]

Following the discovery of the cofactor role for β2GP1, additional candidate cofactors and antigenic targets were identified.[27, 28]

ANTIGENIC TRARGETS OF ANTIPHOSPHOLIPID ANTIBODIES

Major antigens

- β2-glycoprotein
- Prothrombin

Others

- Protein C
- Protein S
- Thrombomodulin
- Annexin V
- High/low molecular weight kininogen
- Factor XI

A number of studies demonstrated that the β2GP1-dependent binding to phospholipids could be used to discriminate between autoimmune aPL and those found in patients following infections, aPL antibodies present in autoimmune diseases are thrombogenic and β2GP1 dependent, as opposed to infection related aPL antibodies, which are thought less likely to be thrombogenic and are β2GP1 independent.[29] The presence of β2GP1 dependent antibodies was shown to be more specific for thrombosis than conventional anti cardiolipin antibodies.

CLASSIFICATION OF ANTIPHOSPHOLIPID THROMBOSIS SYNDROMES

Antiphospholipid thrombosis syndrome associated with anti cardiolipin antibodies is divided into one of six subgroups.[30] Although there appears to be no correlation with the type or the titer of anti cardiolipin antibody and the type of the syndrome, the sub classification of thrombosis and anti cardiolipin antibody patients into these groups is important for therapy.

Type I syndrome

Deep venous thrombosis with or without pulmonary embolus.

Type II syndrome

Coronary artery thrombosis

Peripheral artery thrombosis

Aortic thrombosis

Carotid artery thrombosis

Type III syndrome

Retinal artery thrombosis

Retinal vein thrombosis

Cerebrovascular thrombosis

Transient cerebral ischemic attack

Type IV syndrome

Mixture of types 1 2 3

Type IV patients are rare

Type V (fetal wastage) syndrome

Placental vascular thrombosis

Maternal Thrombocytopenia (uncommon)

Fetal wastage common in first trimester

Fetal wastage can occur in second and third trimester

Type VI syndrome

Anti Phospholipid antibody

No apparent clinical manifestation

Most individuals with serum that reacts positively for anti Phospholipid antibodies do not have systemic lupus, but may have a history of thrombosis. However, the antibodies may also appear transiently after tissue trauma, in infections, and as a response to exposure to certain drugs.

SITUATIONS IN WHICH ANTIPHOSPHOLIPID ANTIBODIES MAY BE DETECTED[31]

Infections

- Acute self-limiting infections
- Syphilis
- Malaria
- HIV infection
- Hepatitis C

Rheumatic and collagen vascular diseases

- Systemic lupus erythematosus
- Systemic sclerosis
- Rheumatoid arthritis
- Temporal arteritis
- Psoriatic arthropathy
- Sjogrens syndrome

Thrombotic disease

- Venous thrombembolic disease
- Peripheral arterial occlusion
- Microvascular thrombosis
- Myocardial infarction and ischaemic heart disease
- After coronary artery bypass graft surgery
- Valvular heart disease
- Renal vascular disease
- Pulmonary hypertension

Disorders of the nervous system and eye

- Thrombotic stroke
- Transient cerebral ischaemia and amaurosis fugax
- Sagittal sinus thrombosis
- Ischaemic optic neuropathy
- Retinal venous occlusion
- Multi infarct dementia
- Chorea
- Guillain-barre syndrome
- Transverse myelitis

Obstetric disorders

- Recurrent abortion
- Fetal growth retardation
- Early, severe pre-eclampsia

With medication

- Phenothiazines
- Procainamide
- Hydralazine
- Phenytoin
- Quinidine

Miscellaneous

- Livedo reticularis
- Autoimmune Thrombocytopenia
- Autoimmune hemolytic anaemia
- Bechet's syndrome
- Sickle-cell disease
- Intravenous drug abuse

ASSOCIATION OF ANTICARDIOLIPIN ANTIBODIES WITH VASCULAR INJURY

Possible Mechanisms

- Endothelial activation
- Accelerated atherosclerosis

- Apoptosis
- Autoimmunity
- Genetic predisposition

ENDOTHELIAL ACTIVATION

Anti cardiolipin have been documented in subendothelial cardiac deposits.[32] and in intimal-medial borders in isolated human atherosclerotic plaques. The mechanism of anti cardiolipin associated vasculopathy includes interaction of endothelial cells with platelets and antiphospholipid, to promote a cascade of reactions yielding recurrent local thrombosis and intimal hyperplasia.

Anticardiolipin auto antibodies prompt a prothrombotic endothelial surface,[33] while the β2GP1 anti cardiolipin antibody complex activates endothelium in vitro.[34]

Antiphospholipid antibody binding to endothelium induces in vitro up-regulation of adhesion molecules, such as intracellular adhesion molecule-1 and extracellular adhesionmolecule-1, stimulated by an autocrine loop of interleukin-1β secretion.[35] Platelet endothelium interaction mediated by anticardiolipin may alter thromboxane A2-prostacyclin balance, leading to enhanced thrombosis and vasoconstriction.[36] Endodthelin-1, which induces vasospasm and arterial occlusion, is released by the endothelium in response to antiphospholipid antibodies.[37]

A mechanism similar to heparin induced thrombocytopenia has also been suggested for anticardiolipin associated vascular occlusion and thrombosis.[38] In addition to endothelial induced thrombosis, intimal hyperplasia plays a major part in vascular occlusions. Endothelin prompted by anticardiolipin, enhances endothelial cell proliferation in vitro.[39]

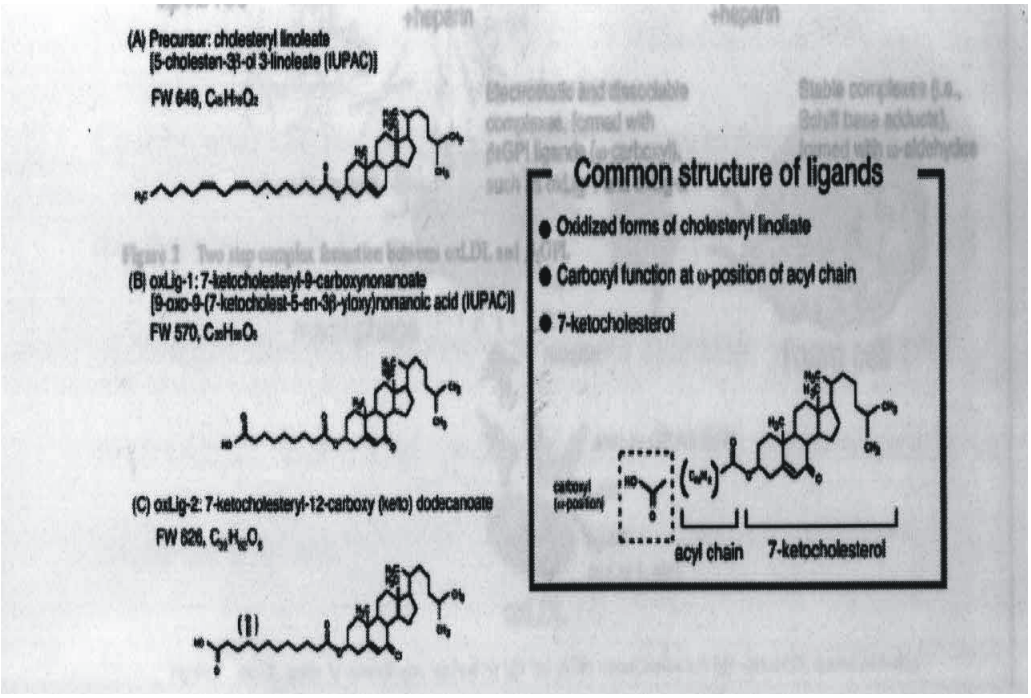
Initial endothelial damage exposes the anionic phospholipids that react with the Phospholipid binding proteins, such as the β2GP1 or prothrombin. The simultaneous binding of aCL to cellular Fc receptor and Phospholipid protein complex induces endothelial platelet interaction resulting in thrombosis.

ACCELERATED ATHEROSCLEROSIS

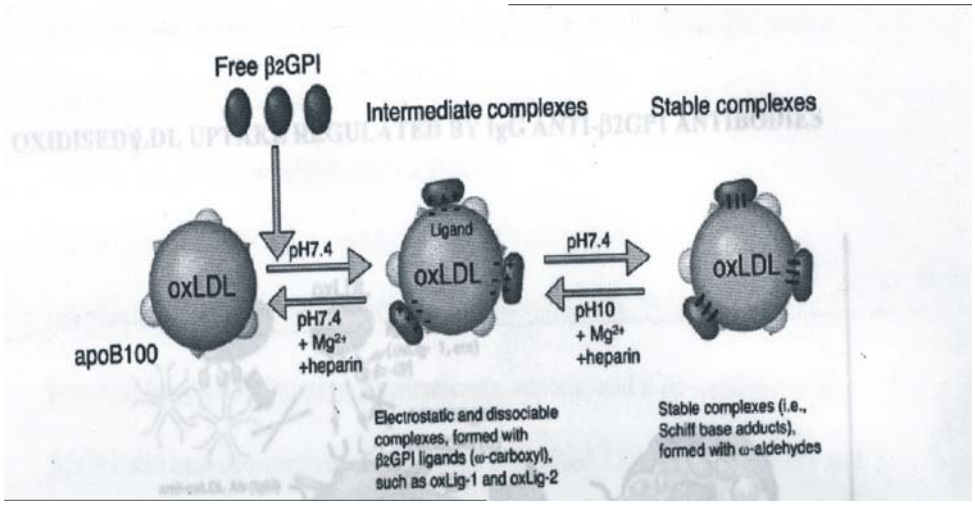
An intriguing possible pathogenic role for aCL in vasculopathy is the cross reaction with oxidized low density lipoprotein antibodies. Oxidized LDL is the principal lipoprotein found in atherosclerotic lesions, and it co-localizes with β2GP1 and immunoreactive lymphocytes.[40] Oxidized LDL binds to β2GP1 and that these complexes can be found in the blood stream of patients with various autoimmune and chronic inflammatory diseases such as the systemic lupus Erythematosus, chronic renal disease, diabetes mellitus, as well as in patients with cerebrovascular disease.[41]

As phospholipids bear structural resemblance to LDL, aCL may cross react with antioxidised LDL.[42] Each cardiolipin molecule contains four unsaturated fatty acids, highly susceptible to oxidation. Mice sera with high titers of oxidized LDL antibodies, bind cardiolipin effectively only after

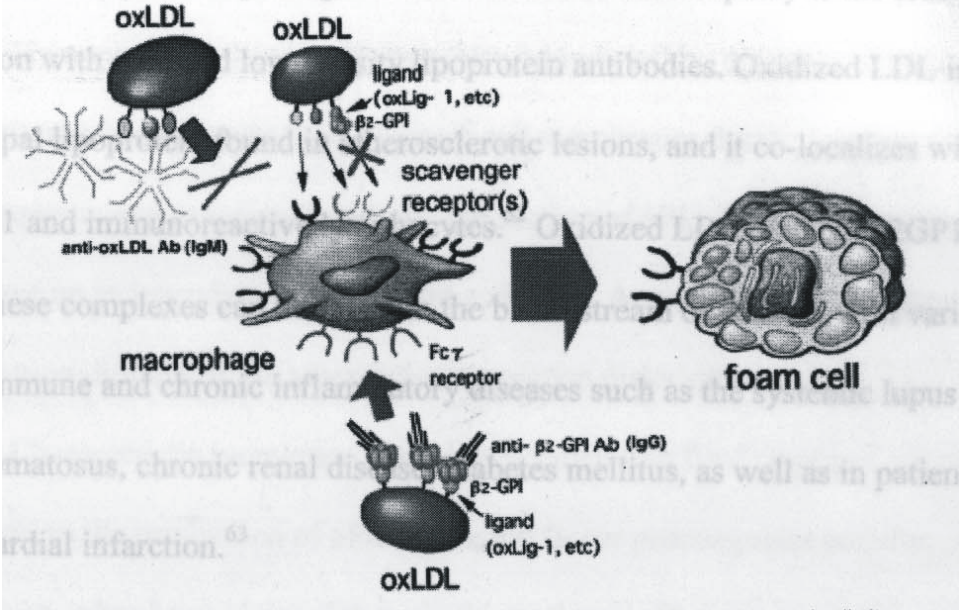
STRUCTURE OF β2 GP1 LIGANDS DERIVED FROM OXIDISED LDL



COMPLEX FORMATION BETWEEN OXIDISED LDL AND β2 GP1



OXIDISED LDL UPTAKE REGULATED BY IgG ANTI- β2GP1 ANTIBODIES



oxidation.Therefore, oxidative events may also play a major path in anticardiolipin formation.[43] On the one hand, oxidized LDL aggravates in vitro the clinical manifestations of antiphospholipid andibody syndrome and on the other hand, atherogenic effect of human lupus sera in vitro may be mediated by LDL-containing immune complexes. LDL may also be involved in anticardiolipin andibodies associated vascular occlusion by inducing a prothrombotic state. LDL itself may be a thrombogenic target of anticardiolipin[44] and raised concentrations of lipoprotein (a) in patients with antiphospholipid antibpdies may inhibit the fibrinolytic sspathway.

APOPTOSIS

Hypercoagulability in patients with antiphospholipid antibodies may also be induced by apoptotic process. Alterations of the Phospholipid phase of cell membranes during late apoptosis are immunogenic and associated with the production of antiphospholipid antibodies. These surface alterations also have an independent procoagulant activity.[45] Apoptotic cells may promote coagulation directly or via athersclerotic plaque dislodgement.

Characteristic membrane blobbing, occurring in final stages of apoptosis may lead to the production of antiphospholipid antibodies and tissue factor procoagulant activity. Moreover, it has been claimed that via this pathway antiphospholipid antibodies may exert their hypercoagulability. Apoptotic inflammatory cells, such as the macrophages and T cells, are found abundantly in ahterosclerotic plaques and may induce plaque instability. Endothelial cell apoptosis may lead to loss of anticoagulant activity and increased leukocyte

and platelet adhesion, resulting in rapid progression of the atherosclerotic and calcification process.[45] On the other hand, antiphospholipid antibodies may enhance apoptosis, with nuclear DNA fragmentation, cell lysis, and membrane disruption.

AUTO IMMUNITY

Pathogenicity of antiphospholipid antibody syndrome depends on specificity, isotype and level of these antibodies .[46] Cross regulatory roles of immunity and autoimmunity have recently emerged in vasculopathic and atherosclerotic processes.[47] A prerequisite for this vascular autoimmunity is a humoral or cellular immune reactivity to self antigens,[48] such as LDL turned immunogenic during oxidation and glycation and prompting T cell mediated immunity.

GENETIC PREDISPOSITION

Most anticardiolipin antibodies are species-specific recognizing only human plasma. They are associated with class 2 major histocompatibility complex and in particular the DQB1 locus and DRw53 and either DR4 (in Caucasians) or DR7 (in Latinos). Genetic factors probably play an important part in the thrombogenic mechanism of antiphospholipids. Therefore, anticardiolipin antibodies may occur in genetically or immunologically susceptible patients after a common infection or after recurrent endothelial results and local thrombosis.

CLINICAL PICTURE OF ATHEROTHROMBOTIC STROKE

In general, the evolution of clinical phenomena in relation to cerebral thrombosis is more variable than that of embolism and hemorrhage. In more than half of our patients, the main part of the stroke (paralysis or other deficit) is preceded by minor signs or one or more transient attacks of focal neurologic dysfunction, or TIAs. In a sense, these herald the oncoming vascular catastrophe. A history of such prodoromal episodes is of paramount importance in establishing the diagnosis of atherothrombotic stroke. Only rarely and for unclear reasons of atherothrombotic stroke. Only rarely and for unclear reasons are embolism and cerebral hemorrhage preceded by a transient neurologic disorder.

In carotid and middle cerebral artery disease, the transient attacks consist of monocular blindness or of hemiplegia, hemianesthesia, or disturbances of speech and language. In the vertebrobasilar system, the prodromal spells most often take the form of episodes of vertigo, diplopia, numbness, impaired vision in one or both visual fields, and dysarthria. Such attacks last from from a few minutes to several hours; in most instances the duration is less than 10 min. The final stroke may be preceded by one or two attacks or a hundred or more brief TIAs, and the stroke may follow the onset of the attacks by hours, days, or less frequently by weeks or months. When there are no prodromal ischemic attacks, one must use other criteria to establish the diagnosis of atherosclerotic thrombosis.

Sl. No.	Clinical Presentation	Percent
1.	Transient ischemic attacks progressing to a major or minor presistent neurologic deficit.	42
2.	Stepwise development of a stroke, with or without transient ischemic attacks.	18
3.	Stroke developing as a single event : Abrupt (hours), with or without fluctuations	11
4.	Slow, gradual (a few days), with or without minor fluctuations	6
5.	Transient ischemic attacks only	14
6.	Development of a limited stroke followed by transient ischemic attacks	9

The thrombotic stroke, whether or not it is preceded by warning attacks, finally develops in one of several ways. Most often there is a single episode but the whole illness evolves over a few hours or less. More characteristic is a "stuttering" or intermittent a day or longer. This a starkly different profile from the abrupt onset of a complete stroke syndrome that characterizes the embolic mechanism. Again, in thrombosis a partial stroke may occur and even recede temporarily for several hours, after which there is rapid progression to the completed stroke – or several fleeting episodes may be followed by a longer one and, hours or a day or two later, by a major stroke. Several parts of the body may be affected at once or only one part, such as a limb or one side of the face, the other parts becoming involved serially in step-like fashion until the stroke is fully developed. Sometimes the deficit is episodic; spells of weakness or involuntary movement of a hand or arm or dimness of vision, lasting 5 to 10 min. occur spontaneously or are brought on by standing or walking. Each of the

transient attacks and the abrupt episodes of progression reproduces the profile of the stroke in miniature. The principle of intermittency seems to characterize the thrombotic process from beginning to end.

As frequent as the modes of onset outlined above, and most characteristic of atherombotic events, is the occurrence of the stroke during sleep; the patient awakens paralyzed, either during the night or in the morning.

Most deceptive of all are the relatively few patients in whom the neurologic disorder has evolved over several days or even longer, in a slow, gradual fashion ("slow stroke"). One's first impulse is to make a diagnosis of brain tumor, abscess, or subdural hematoma. This error can usually be avoided by a careful analysis of the course of the illness, which will disclose an uneven, saltatory progression; if the clinical data are incomplete, observation for a few days makes the stroke profile more apparent. Actually there are a few cases – and these are usually instances of pure motor hemiplegia – in which the evolution of a thrombotic stroke is evenly progressive over a period of days.

In addition to these several modes of evolution of atherothrombotic stroke, thrombotic stenosis or occlusion of certain large vessels may lead instead to the generation of embolic fragments (artery-toartery embolus), thereby precipitating a new stroke in a region distal to the occlusion. This is most likely to occur during the period of clinical fluctuation and active thrombus formation. The most common occurrence of artery-to-artery embolism is with carotid artery thrombosis, the embolus passing to branches of the ipsilateral middle or anterior cerebral artery. With atherothrombotic blockage of the vertebral or lower basilar artery, the embolus originates in the occluded vessel but then proceeds to lodge in the posterior cerebral artery or

the top of the basilar artery. In most of the cases of this nature, there are additional telltale signs of slight pontine strokes (dysarthria, diplopia), presumably from the transient occlusion of the mouths of several small penetrating arteries as the embolus moves upward ("stop-and-go" or "traveling" embolus syndrome).

CAROTID ARTERY OCCLUSION

Common carotid occlusion accounts for less than 1 percent of cases of carotid artery syndrome – the remainder being due to disease of the internal carotid artery itself. Nevertheless, the common carotid can be occluded by an atheromatous plaque at its origin, more often on the left side. Atherosclerotic stenosis or occlusion of the midportion of the common carotid may also occur years after radiation therapy for laryngeal or other head and neck cancer. If the bifurcation is patent, few if any symptoms may result, in some cases because retrograde flow from the external carotid maintains internal carotid flow and perfusion of the brain.

The syndrome caused by common carotid occlusion are identical to those of its internal branch. The clinical manifestations of atherosclerotic thrombotic disease of internal carotid artery are among the most variable of any cerebrovascular syndrome. Unlike other cerebral vessels, the internal carotid artery is not an end vessel. In most individuals it is in continuity with the vessels of the circle of Willis and those of the orbit, and no part of the brain is completely dependent on it. Therefore occlusion, which occurs most frequently in the first part of the internal carotid artery (immediately beyond the carotid bifurcation), is often silent (30 to 40 percent of cases).

Headache – located as a rule, above the eyebrow – may occur with thrombosis or embolism of the carotid artery, but cranial pain is not invariable. The headache sometimes associated with occlusion of the middle cerebral artery tends to be more lateral, at the temple; that of posterior cerebral occlusion is in or behind the eye.

When the circulation of one carotid artery has been incompletely compromised, reducing blood flow in both the middle and anterior cerebral territories, the zone of maximal ischemia lies between the two vascular territories ("cortical watershed") or, alternatively, in the deep portions of the hemisphere, between the territories of the lenticulostriate branches and the penetrating vessels from the convexity ("internal or deep watershed"). The infarction in the first instance occupies a region in the high parietal and frontal cortex and subcortical white matter. Its size depends upon the adequacy of collateral vessels. The weakness that results tends to involve the shoulder and hip more than the hand and face. With long-standing carotid stenosis, the carotid watershed zone tends to shift downward toward the perisylvian portions of the middle cerebral artery territory, even to the extent of affecting facial movement or causing a motor aphasia. The frequent sparing of the posterior part of the hemisphere is reflected in the low incidence of Wernicke types of aphasia and of homonymous hemianopia. With impaired perfusion of the so-called internal watershed, infarctions of varying size are situated in the subfrontal and subparietal portions of the centrum semiovale.

The internal carotid artery nourishes the optic nerve and retina as well as the brain. For this reason, transient monocular blindness occurs prior to the onset of stroke in 10 to 25 percent of cases of symptomatic carotid occlusion.

Yet central retinal artery ischemia is a relatively rare manifestation of carotid artery occlusion, presumably because of efficeint collateral supply in the globe. With severe atherosclerotic stenosis at the level of the carotid sinus, with or without a superimposed thrombus, auscultation frequently discloses a bruit, best heard with the bell of the stethoscope. If the bruit is loudest at the angle of the jaw, the stenosis usually lies at the proximal internal carotid; if heard lower in the neck, it is in the common carotid or subclavian artery. Rarely, stenosis in vertebral arteries or vascular malformations at the base of the brain may produce bruits posteriorly in the neck.

The duration and quality of the bruit are relevant – bruits that extend into diastole and are high-pitched are almost invariably associated with a tight stenosis (lumen < 1.5 mm). An additional though infrequent sign of carotid occlusion is the presence of a bruit over the opposite carotid artery, heard best by placing the bell of the stethoscope over the eyeball (ocular bruit). Pulsation may be palpably reduced or absent in the common carotid artery in the neck, in the external carotid artery in front of the ear, and in the internal carotid artery in the lateral wall of the pharynx, but these are among the least dependable signs of carotid disease.

DIAGNOSIS

In the laboratory investigation of atherothrombotic infarction, one may employ non-invasive techniques.

- **Ultrasonography** : will reveal with fair accuracy the cervical and intracranial segments of the internal carotid and vertebrobasilar arteries.

- **Computed Tomography** : CT imaging is mandatory for proper initial evaluation in all cases of stroke to categories them into either ischemic (or) haemorrhagic origin.

    1. To confirm diagnosis (Haemorrhage can be detected immediately whereas it may take two days for infarcts to the detected).

    2. To decide the line of management (to decide on therapy with anticoagulants (or) anti platelet drugs).

    3. To identify the presence of underlying tumour, haematoma (or) vascular malformation which can stimulate stroke.

CT FINDINGS IN CEREBRAL INFARCTION

Stage of infarct	CT findings
Hyper acute (< 12 hours)	Normal (50 to 60%), hyperdense artery (25 to 50%), obscuration of lentiform nuclei
Acute (12 to 24 hours)	Low density basal ganglia, loss of grey-white matter interface (insular ribbon sign), sulcal effacement
1 to 7 days	Mass effect, wedge shaped low density area involving white and gray matter, haemorrhagic transformation, gyral enhancement
1 to 8 weeks	Contrast enhancement persists, mass effect resolves
Months to years	Encephalomalacic change, volume loss, rarely calcification.

- **Magnetic Resonanace Imaging :** MRI is more sensitive to ischemic brain damage than is the CT scan. While the later reveals hemorrhage immediately after it occurs, softened tissue cannot be seen until several

days have elapsed. On the other hand, MRI reveals ischemic damage with in a few hours, in both white and grey matter, and diffusion – weighted MRI techniques do so even earlier. The various MRI sequences are able to distinguish with reasonable clarity the age of cerebral infarction.

- **Opthalmic dynamometry** : A diastolic retinal pressure of less than 20 mmHg. Usually means that the common or internal carotid artery is occluded.

- **Blood Sugar**

- **Serum Cholesterol, Triglycerides .**

- **ECG (Electrocardiography)** : Reflecting a previous myocardial infarction or left ventricular hypertrophy.

- **EEG (Electroencephalogram)** : It is of value in distinguishing large strokes from lacunes.

MATERIALS AND METHODS

This study was an observational study conducted on a total of 35 patients admitted in the Intensive Medical Care Unit and General Medical Wards at Government Stanley Hospital, Chennai. The period of study was about 8 months (From July 2006 to March 2007).

Selection Criteria

Patients with the age of ≤ 55 years of either sex who admitted with the acute onset of focal neurological deficit with the CT brain showing infarction were included in the study.

The prevalence of positive ELISA antiphospholipid antibody titer increases with age. Most data deal with anticardiolipin antibodies, which have been reported in 12 – 52% of apparently healthy elderly aged more than 65 years [49,50]. Hence, a cutoff age of 55 was considered in the study group.

35 age and sex matched apparently healthy persons served as control. Conventional cerebrovascular risk factors such as hypertension, diabetes, smoking and alcohol were determined by a thorough history taking. The blood samples are taken on the 2nd day of hospitalization after getting the consent from the patients. The quantitative measurement of IgG and IgM class autoantibodies against cardiolipin in human serum (or) plasma was determined by an indirect solid phase enzyme immunoassay (ELISA).

Data were analyzed using Pearson Chi square test and Yates corrected chi square test.

NAME OF THE TEST

It is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG and IgM class autoantibodies against cardiolipin in human serum or plasma.

PRINCIPLE

Highly purified cardiolipin is bound to micro wells saturated with β2 glycoprotein 1. Antibodies to these antigens, if present in diluted serum, bind in the micro wells. Washing of the micro wells removed unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti human IgG and IgM immunologically bind to the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing the micro wells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration of IgG and IgM antibodies present in the original sample.

STORAGE AND STABILITY

- Store the kit at 2 – 8^{o}C.
- Keep micro plate wells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit
- Do not expose the test reagents to heat, sun or strong light during storage and usage.
- Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2 – 8^{o}C.

MATERIALS REQUIRED

Equipment

- Micro plate reader capable of end point measurements at 450 nm.
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Laboratory timing device
- Data reduction software

Preparation of reagents

- Distilled or deiodinised water
- Graduated cylinder for 100 and 100 ml
- Plastic container for storage of the wash solution.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum by centrifugation.
- Test serum should be clear and non-hemolysed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.

- Specimens may be refrigerated at 2 – 8°C for up to 5 days or stores at –20°C for up to six months.

- Avoid repeated freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.

- Testing of heat-inactivated sera is not recommended.

PREPARATION OF THE REAGENTS

Preparation of the sample buffer

Dilute the contents of each vial of the sample buffer concentrate with distilled or deiodinised water to a final volume of 100 ml prior to use. Stores refrigerated: stable at 2 – 8°C for at least 30 days after preparation of until the expiration date printed on the label.

Preparation of the wash solution

Dilute the contents of each vial of the buffered wash solution concentrate with distilled or deiodinised water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2 – 8°C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples 1:100 with sample buffer before assay. Therefore, combine 10µl of sample with 990µl of sample buffer in a polystyrene tube. Mix well. Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of micro plate modules to accommodate controls and pre diluted patient samples.

2. Pipette 100µl of calibrators, controls and pre diluted patient samples in duplicate into the wells.

3. Incubate for 30 minutes at room temperature (20 – 28^{o}C).

4. Discard the contents of the micro wells and wash 3 times with 300µl of wash solution.

5. Dispense 100µl of enzyme conjugate into each well.

6. Incubate for 15 minutes at room temperature

7. Discard the contents of the micro wells and wash 3 times with 300µl of was solution.

8. Dispense 100µl of TMB (3,3', 5,5' –tetramethyl-benzidine) substrate solution into each well.

9. Incubate for 15 minutes at room temperature.

10. Add 100µl of stop solution to each well of the modules and incubate for 15 minutes at room temperature.

11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600 – 690 nm is recommended.

12. The developed color is stable for at least 30 minutes. Read optical densities during this time.

INTERPRETATION OF RESULTS

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the anti-cardiolipin test.

ANTI CARDIOLIPIN ANTIBODIES

	IgG (GPL U/ml)	**IgM (MPL U/ml)**
NORMAL	< 10	< 7
POSITIVE	≥ 10	≥7

Quality control

This test is only valid if the optical density at 450 nm for positive control and negative control as well as for the calibrator A and F complies with the respective range indicated on the quality control certificate enclosed to each test kit. If any of these criteria is not met, results are invalid and the test should be repeated.

Calculation of the results

For anti cardiolipin IgG and IgM, a 4 parameter fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed spline approximation and log-log coordinates are also suitable.

Recommended Lin-Log plot

First calculate the averaged optical densities for each caliber well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight-line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

OBSERVATIONS AND RESULTS

In the present study, among the 35 cases, the age distribution ranged from 35 – 56 years. Since age is a major risk factor for cerebrovascular disease, the cases were subdivided into two groups with 45 as the line of demarcation between them. Acute ischemic stroke occurred predominantly in males when compared to females in our study.

TABLE 1

GENDER DISTRIBUTION OF THE STUDY POPULATION

	MALE	FEMALE
STUDY GROUP (35)	**30 (85.7%)**	**5 (14.3%)**

TABLE 2

AGE DISTRIBUTION OF THE STUDY POPULATION

	AGE > 45	AGE <=45
STUDY GROUP	**19 (54.3%)**	**16(45.7%)**

The presence of the conventional cerebrovascular risk factors in the study population and its distribution was analysed. Of the four risk factors (Hypertension, Diabetes Mellitus, Smoking, Alcohol) that were considered, smoking and Alcohol were widely prevalent in the study group.

TABLE 3

DISTRIBUTION OF DIABETES MELLITUS AMONG THE CASES

	DIABETICS	NON-DIABETICS
STUDY GROUP	9 (25.7%)	26(74.3%)

TABLE 4

DISTRIBUTION OF HYPERTENSION AMONG THE CASES

	HYPERTENSIVES	NON-HYPERTENSIVES
STUDY GROUP	10(28.6%)	25(71.4%)

TABLE 5

DISTRIBUTION OF SMOKING AMONG THE CASES

	SMOKERS	NON-SMOKERS
STUDY GROUP	23(65.7%)	12(34.3%)

TABLE 6

DISTRIBUTION OF THE β2GP1 DEPENDENT ANTICARDIOLIPIN ANTIBODY POSITIVITY AMONG THE CASES AND CONTROLS

	ANTIBODY POSITIVITY	ANTIBODY NEGATIVITY
STUDY GROUP	10(28.6%)	25(71.4%)
CONTROL GROUP	2(5.7%)	33(94.3%)

Error! $X^2 = 6.44$ p = 0.01 (Statistically significant)

The antibody positivity was compared between the cases and the controls. The β2 glycoprotein1 dependent anticardiolipin antibodies were found to be positive in 10 patients in the study group whereas 2 people tested positive in the control group. The antibody positivity was statistically significant in the study group.

TABLE 7

ISOTYPE DISTRIBUTION OF ANTIBODY POSITIVITY IN CASES AND CONTROLS

	STUDY GROUP	CONTROL GROUP
IgG POSITIVITY	6	0
IgM POSITIVITY	3	2
IgG & IgM POSITIVITY	1	0

Isotype distribution of anticardiolipin antibodies namely IgG and IgM was studied in both the groups. IgG was the predominant isotype in the study group. A total of 6 patients were found to have IgG antibody positivity. 3 patients showed IgM positivity while one had elevation of both the isotypes.

TABLE 8

IgG β2GP1 ANTICARDIOLIPIN ANTIBODY IN CASES AND CONTROLS

	IgG POSITIVITY	IgG NEGATIVITY
STUDY GROUP	7	28
CONTROL GROUP	0	35

$X^2 = 7.78$ p = 0.005 (Statistically significant)

The significance of the antibody isotypes was then analysed across both the groups. It was found that the isotype IgG positivity was statistically significant in the study group when compared to the control group. The IgM positivity did not show any significant difference between the two groups.

TABLE 9

IgM β2GP1 ANTICARDIOLIPIN ANTIBODY IN CASES AND CONTROLS

	IgM POSITIVITY	IgM NEGATIVITY
STUDY GROUP	4	31
CONTROL GROUP	2	33

Error!

X^2 Y=0.73 p = 0.39 (Not significant)

The conventional risk factors were then correlated with antibody positivity. Diabetes Mellitus was the only factor that was significantly observed in the study group.

TABLE 10

DIABETES MELLITUS AND ANTICARDIOLIPIN POSITIVITY

	ANTIBODY POSITIVITY	ANTIBODY NEGATIVITY
DIABETES MELLITUS	**5**	**4**
NON-DIABETES MELLITUS	**5**	**21**

TABLE 11

HYPERTENSION AND ANTICARDIOLIPIN ANTIBODIES

	ANTIBODY POSITIVITY	ANTIBODY NEGATIVITY
HYPERTENSIVES	**4**	**6**
NON-HYPERTENSIVES	**6**	**19**

$X^2 = 0.90$ p = 0.34 (Not significant)

TABLE 12

SMOKING AND ANTICARDIOLIPIN ANTIBODIES

	ANTIBODY POSITIVITY	ANTIBODY NEGATIVITY
SMOKERS	5	18
NON-SMOKERS	5	7

$X^2 = 1.53$ $p = 0.21$ (Not significant)

TABLE 13

SEX AND ANTICARDIOLIPIN ANTIBODIES POSITIVITY

	ANTIBODY POSITIVITY	ANTIBODY NEGATIVITY
MALE	8	22
FEMALE	2	3

$X^2 = 0.37$ $p = 0.54$ (Not significant)

TABLE 14

AGE AND ANTICARDIOLIPIN POSITIVITY

	ANTIBODY POSITIVITY	ANTIBODY NEGATIVITY
AGE > 45	5	14
AGE $\leq$ 45	5	11

$X^2 = 0.10$ $p = 0.74$ (Not significant)

DISCUSSION

Anti cardiolipin antibodies are most commonly found in patients with systemic lupus erythematosus and antiphospholipid antibody syndrome. However, they are not specific to systemic lupus erythematosus and antiphospholipid antibody syndrome and may occur in normal subjects, patients with other autoimmune disorders, malignancy, HIV infection and drugs[51,52] . Levine reported the association between anticardiolipin antibodies and stroke. It has been well established in patients with primary APL syndrome and systemic lupus erythematosus associated secondary APL syndrome, but a growing body of evidence supports an association in unselected patients as well[53].

The cumulative prevalence rates for increased anticardiolipin antibody titer in ischemic stroke patients were relatively high (In all ages 17%, age < 50 years 21%)[54].

Reyes H states that APL antibodies have been found in upto 14% of normal subjects[55,56,57]. Nagaraj et al in a study of 60 cases of young stroke found elevated anticardiolipin in 23% of patients[58]. Some studies have found widely varying frequencies of anticardiolipin antibodies in patients with stroke with the prevalence from 1 to 50%.

Mehndiratta and Bhattacharya reported that the prevalence of antiphospholipid antibodies in healthy people is 4.3%, whereas in stroke patients it is about 1 to 46% depending upon the design and criteria of studies and the age distribution of patients[59].

A number of studies demonstrated that the β2GP1 dependent binding to phospholipids could be used to discriminate between autoimmune anticardiolipin and those found in patients following infections. Anticardiolipin antibodies present in autoimmune diseases are thrombogenic and β2GP1 dependent, as opposed to infection related anticardiolipin antibodies, which are thought less likely to be thrombogenic and are β2GP1 independent. So, the presence of β2GP1 antibodies was shown to be more specific for thrombosis than conventional anticardiolipin antibodies.

In the population that we examined, a prevalence of 28.6% β2GP1 dependent anticardiolipin positive patients were found. This figure is significantly higher than the 5.7% observed in the control group ($p=0.01$). However, IgM β2GP1 dependent anticardiolipin antibodies were not significantly increased in the study group.

Here, we found a notable, although not significant difference in the frequencies of β2GP1 dependent anticardiolipin antibodies between males and females (26.6%) verses 40%, respectively. But females contributed only about 14.3% of the study population.

When we divided our patients into two groups, based on age (group 1 age > 45, group II age ≤ 45) there was no significant difference in the frequencies of β2GP1 dependent anticardiolipin antibodies between them.

We found significantly higher frequencies of β2GP1 dependent anticardiolipin antibodies among the patients with diabetes mellitus. However, we did not find any significant association between β2GP1 dependent anticardiolipin antibodies positivity and other conventional cerebrovascular risk factors like Hypertension and smoking.

There are a good number of studies that looked into the prevalence of anticardiolipin antibodies of isotypes IgG and IgM in patients with ischemic stroke and its relationship with other risk factors.

Robin, L. Brey et al performed a nested case control study examining anticardiolipin as a risk factor for ischemic stroke and myocardial infarction by using stored frozen sera obtained from subjects enrolled in Honolulu heart programme and followed up for 20 years. The β2GP1 dependent anticardiolipin of the class IgG was significantly associated with both ischemic stroke and myocardial infarction[60].

D'olhaberriague et al reported significantly increased odds of anticardiolipin positivity in ischemic stroke patients compared with patients with the other neurological diseases, suggesting that the antibodies are specific to stroke[61].

Tannel et al reported that anticardiolipin IgG titers are associated with the presence of multiple stroke risk factors such as age > 65 years, atrial fibrillation, valvular heart disease, congestive heart failure[62].

Brey et al demonstrated an association between β2GP1 dependent anticardiolipin and incidence of ischemic stroke. Their results showed that patients with IgG β2GP1 dependent anticardiolipin had a two fold increase in odds of stroke with in 15 years of follow up when compared with anticardiolipin negative individuals. This study also gives further evidence for the role of anticardiolipin as an independent risk factors for ischemic stroke in the general population[63].

Levine suggested that IgG is the anticardiolipin most commonly seen with antiphospholipid antibody positive stroke. Young stroke patients have higher levels of antiphospholipid antibodies and multiple infarctions than general stroke population[64]. Finazzi G and Branacaccio found the presence of high titre of anticardiolipin immuno reactivity mainly IgG isotype but possible IgM also correlates with an increased risk of thrombosis. A study which was undertaken by Tuhrim et al showed that the elevated anticardiolipin antibody titer is a stroke risk factor in a multi ethnic population independent of isotype (or) degree of positivity. He also states that anticardiolipin antibodies conferred a four fold increased risk of ischemic stroke[65].

Few studies have shown increased incidence of recurrent ischemic stroke in patients with high anticardiolipin antibody titer positivity. Patients with elevated IgG anticardiolipin levels had the shortest times to subsequent thrombo occlusive events, mainly represented by cerebral infarction often occurring within the first year of follow up supporting that IgG anticardiolipin represented a risk factor for recurrent stroke.

A recent study of APASS (Antiphospholipid Antibodies and Stroke Studies) found that a single anticardiolipin antibody value $\geq$ 10 GPL units at the time of initial ischemic stroke was a significant independent risk factor for stroke. When titer is high $\geq$ 40 GPL units recurrence is common[66].

Since anticardiolipin antibodies are a novel modifiable risk factors for stroke, some of the studies has been undertaken to know its therapeutic significance.

Panagariya et al analysed 12 stroke patients with anticardiolipin antibody positivity and found that both the IgG and IgM class anticardiolipin antibodies were raised equally. Therapeutic option in patients with stroke and increased anticardiolipin antibodies include antithrombotics and immune based treatment.

In his series, patients with first episode of stroke were put on aspirin (or) ticlopidine. Patients with recurrent events were put on oral anticoagulants. All patients recovered to normal functional capacity and did not have recurrence[67].

APASS study states that nevertheless in those with persistently high titer of anticardiolipin (or) persistently positive lupus anticoagulant and some features of the antiphorpholipid antibody syndrome there does seem to be an association between anticardiolipin antibodies and stroke. In such patients anticoagulation with an INR $\geq$ 3 seems to be more effective than low intensity warfarin (or) aspirin in preventing recurrent thrombosis.

Mehndiratta and Bhattacharrya [59] stated that there are no clear recommendations about the duration of the therapy but consensus could favors the lifelong treatment with antiplatelet drugs (or) anticoagulants to prevent recurrences in stroke patients with antiphospholipid antibodies positivity.

CONCLUSION

- The prevalence of β2 glycoprotein1 dependent anticardiolipin antibodies of isotyes IgG/IgM was found to be 28.6% ($p = 0.01$).
- IgG β2 glycoprotein1 dependent anticardiolipin antibodies was the most relevant isotype associated with acute ischemic stroke ($p = 0.005$).
- Patients with Diabetes Mellitus had a higher frequency of β2 glycoprotein dependent anticardiolipin antibodies ($p = 0.04$).
- β2 glycoprotein1 dependent anticardiolipin antibodies did not show a significant association with other conventional risk factors like Hypertension, smoking, alcohol and age.

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PROFORMA

"PREVALENCE OF β2 GLYCOPROTEIN 1 DEPENDENT ANTICARDIOLIPIN ANTIBODIES IN ACUTE ISCHEMIC STROKE"

Name :

Age :

Sex :

Clinical Presentation

Risk Factors

Diabetes Mellitus :

Systemic Hypertension :

Smoking :

Alcohol :

Heart Diseases :

Obesity :

Others

Investigations

Hb :

ESR :

Platelet count :

B-Sugar :

S-Cholesterol :

CRP :

ANA :

Rh-Factor :

ECG :

ECHO :

CT Brain :

β2 GP1 Dependent Anticardiolipin Antibodies

- IgG :

- IgM :

MASTER CHART

STUDY GROUP										CONTROL GROUP									
Sl. No.	Name	Age	Sex	Diabetes Mellitus	Hypertension	Smoking	Alcohol	IgG	IgM	Sl. No.	Name	Age	Sex	Diabetes Mellitus	Hypertension	Smoking	Alcohol	IgG	IgM
1	VEERAMANI	0	1	0	1	1	1	1	0	1	SUBRAMANIYAN	1	1	0	0	0	0	0	0
2	KHADAR	0	1	1	1	1	1	1	0	2	VADIVELU	0	1	0	0	1	0	0	0
3	KUPPAN	1	1	1	1	1	1	1	0	3	GODANDARAMAN	0	1	0	0	0	0	0	0
4	PONNUSAMY	0	1	0	0	0	0	0	0	4	RAGHAVAN	0	1	0	0	0	1	0	0
5	AKRAM	0	1	0	0	1	0	0	1	5	MUNUSAMY	0	1	0	0	0	0	0	0
6	GOVINDAN	1	1	0	0	1	1	0	0	6	KUZHANTHIVELU	1	1	0	0	0	0	0	0
7	VASU	1	1	1	0	0	1	1	1	7	SIVAGURU	1	1	0	0	1	1	0	0
8	IRUDAYAN	1	1	0	1	1	1	0	0	8	KANNIYAPPAN	0	1	0	0	1	0	0	0
9	KALI	0	1	0	0	1	1	0	0	9	PANNERSELVAN	0	1	0	0	0	0	0	0
10	ELUMALAI	1	1	1	0	1	1	0	0	10	NATARAJAN	1	1	0	0	0	0	0	0
11	ANTONY	0	1	0	0	1	1	0	0	11	PALANISAMY	0	1	0	0	0	1	0	0
12	MANI	1	1	0	0	1	1	0	0	12	SENDHILNADHAN	0	1	0	0	0	0	0	0
13	BASHA	1	1	0	1	1	1	0	0	13	MANIKANDAN	1	1	0	0	0	0	0	0
14	RAVI	1	1	0	1	1	0	0	0	14	VENKATESAN	1	1	0	0	0	0	0	0
15	SOUKANTHALI	0	1	0	0	1	1	0	0	15	ARUNAGIRI	1	1	0	0	1	0	0	0
16	PICHAIAN	0	1	1	0	1	1	0	0	16	KARUNANITHI	0	1	0	0	1	1	0	0
17	KANNAN	1	1	0	0	1	1	0	0	17	ANBHAZHAGAN	0	1	0	0	0	0	0	0
18	MUNUSAMY	0	1	0	0	1	1	0	0	18	MURUGESAN	1	1	0	0	0	0	0	0
19	SHANMUGAM	0	1	0	1	1	1	0	1	19	NARAYANAN	1	1	0	0	0	0	0	0
20	MUTHUKRISHNAN	0	1	0	1	0	1	0	0	20	RAJA	0	1	0	0	0	0	0	0
21	MANI	1	1	0	0	0	0	0	1	21	KALAIARASAN	1	1	0	0	1	0	0	0
22	RAMASAMY	0	1	1	0	1	1	0	0	22	MANIKKAM	0	1	0	0	0	1	0	0

STUDY GROUP										CONTROL GROUP									
Sl. No.	Name	Age	Sex	Diabetes Mellitus	Hypertension	Smoking	Alcohol	IgG	IgM	Sl. No.	Name	Age	Sex	Diabetes Mellitus	Hypertension	Smoking	Alcohol	IgG	IgM
23	JAYAVEL	1	1	0	0	1	1	0	0	23	RAJENDIRAN	0	1	0	0	0	0	0	0
24	SUNDARAJAN	1	1	0	0	0	1	0	0	24	SUBBIAH	1	1	0	0	1	0	0	0
25	SAMIKANNU	0	1	0	0	1	0	0	0	25	BASHEER AHMED	1	1	0	0	0	0	0	0
26	SIVARAJAN	0	1	0	0	1	0	0	0	26	JAYAKUMAR	1	1	0	0	0	1	0	0
27	RAJA	1	1	0	0	0	1	1	0	27	SARAVANAN	0	1	0	0	0	1	0	0
28	SUBRAMANI	0	1	0	0	1	1	0	0	28	JOHNBABU	0	1	0	0	0	0	0	0
29	MUNIVEL	0	1	0	0	0	1	0	0	29	SUNDERESAN	1	1	0	0	1	0	0	0
30	RAGAVEL	1	1	1	0	1	0	0	0	30	ASHOGAN	0	1	0	0	0	0	0	0
31	KASTHURI	0	0	0	1	0	0	0	0	31	BHAVANI	1	0	0	0	0	0	0	0
32	NAVANEEDHAM	0	0	0	0	0	0	0	0	32	RAJAMMAL	0	0	0	0	0	0	0	1
33	BALAMMAL	1	0	1	0	0	0	1	0	33	SULOCHANA	0	0	0	0	0	0	0	0
34	SANJEEVAMMAL	0	0	1	0	0	0	1	0	34	ELLAMMAL	0	0	0	0	0	0	0	0
35	NAZEEMA	1	0	0	1	0	0	0	0	35	RANI	1	0	0	0	0	0	0	0

	1	0
Age	≤ 45 Yrs	> 45 yrs
Sex	Male	Female
Diabetes Mellitus/Hyper-tension/Smoking/ Alcohol	Positive	Negative
IgG	≥ 10 GPL	< 10 GPL
IgM	≥ 7 MPL	< 7 MPL